Published in final edited form as:

Wiley Interdiscip Rev Membr Transp Signal. 2012 ; 1(5): 641–653. doi:10.1002/wmts.43.

“mGlu Receptors in the Retina” - WIREs Membrane Transport and Signaling

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Abstract

Glutamate, a key neurotransmitter in the vertebrate retina, acts via ionotropic and metabotropic receptors. Retina expresses mRNA for all metabotropic glutamate receptors and proteins for all but mGluR3. Every retinal cell class expresses one or more of these receptors. In general, these receptors are present presynaptically and serve to modulate synaptic transmission. While mGluRs on the photoreceptor terminal act as autoreceptors to titer glutamate levels, those on horizontal cell processes seem to shape the light response. Similarly, autoreceptors on bipolar axon terminals modulate glutamate release and the receptors on amacrine and ganglion cells modulate feedforward signals by modulating K^+ or Ca^{2+} current to fine tune light responses. Since most of the mGluR sub-types are present in amacrine and ganglion cells that belong to many cell types, the pathways downstream of mGluRs are highly diverse with primarily modulatory effects. An exception to most mGluRs which have modulatory function is mGluR6 because it plays a key role in the feedforward transmission from photoreceptors to ON bipolar cells and is also required for the correct localization of the synaptic proteins in the dendritic tips. In humans, mutations in the gene encoding mGluR6 cause autosomal recessive night blindness. In addition, mGluRs appear to play a trophic role in development and after retinal damage, suggesting potential future therapeutic implications.

In retina, glutamate serves as the main, or perhaps only, feedforward neurotransmitter. It is released by photoreceptors onto horizontal and bipolar cells and by bipolar cells onto amacrine and ganglion cells. This feedforward pathway from photoreceptors to bipolar cells to ganglion cells is heavily modulated by horizontal and amacrine cells that use numerous molecular mechanisms to provide feedback onto the various pathways. It is also modulated significantly by autoreceptors that are primarily metabotropic glutamate receptors. Eight metabotropic glutamate receptors are known, and all proteins but one appear to be expressed in the retina (Table 1 and Fig. 1). In general, these receptors are found presynaptically and serve to modulate synaptic transmission. However, one receptor, mGluR6, stands out because it plays a key role in the feedforward transmission from photoreceptors to a particular class of bipolar cells. Here we will review the localization and possible functions of the metabotropic glutamate receptors found in each class of retinal cell. Summary of functional pathways is given in Fig. 2.

1. The first synaptic layer: synapses from photoreceptors to second order cells

There exist two classes of photoreceptor: rods that are responsible for night vision, and cones that are responsible for daylight vision. These photoreceptors transduce light energy into voltage changes and transmit this information forward to horizontal cells and to two classes of bipolar cells, the ON class that depolarizes to light and the OFF class that hyperpolarizes to light. The number of horizontal cell or bipolar cell types vary greatly between species, but the basic structure of the synaptic complex remains similar: a presynaptic photoreceptor terminal houses synaptic ribbons that define the synaptic release

sites, and each such site lies presynaptic to a triad. The triad consists of two lateral elements belonging to horizontal cell processes, and one central element belonging to the dendrite of an ON bipolar cell (Fig. 1). Dendrites of OFF bipolar cells contact the terminals some 0.5–1 μm away from the ribbon site. While the photoreceptor terminal is clearly presynaptic and the bipolar dendrite is clearly postsynaptic, the horizontal cell processes are both pre- and postsynaptic.

1.1 Presynaptic mGluRs in photoreceptors

A number of studies report that metabotropic glutamate autoreceptors modulate transmitter release from photoreceptors. However, the details differ somewhat, especially between higher and lower vertebrates. In higher vertebrates, studies have concentrated on rat rods. Calcium imaging from isolated rods has shown that glutamate, as well as the specific agonists of group III mGluRs [L-2-amino-4-phosphonobutyrate (L-AP4) and L-serine-O-phosphate], but not the agonists for other glutamate receptors, decreases intracellular calcium concentration¹. This mGluR-evoked effect depends on extracellular calcium and is mediated by a pertussis toxin-sensitive G-protein, possibly transducin (G_t), whose main function is to mediate phototransduction in the outer segment. The immediate down-stream effector for this G-protein is unknown, but clearly a decrease in calcium concentration will tend to reduce glutamate release. Understanding the exact function of such feedback in image processing requires understanding its time constant and sensitivity. Nonetheless, it is clear that this feedback will help to regulate the glutamate concentration in the cleft and thus may be crucial for reducing toxicity.

In lower vertebrates, studies have been performed on several species, and the key finding from higher vertebrates, that group III mGluRs provide autofeedback in photoreceptors, holds true for these species as well. A nice study performed in newt retina showed that mGluR group III agonists shift the **cone** calcium activation curve to higher voltages via a G-protein cascade, and thereby reduce the presynaptic calcium current. This in turn reduces the light-off-activated inward current in second order cells (tested in horizontal cells and OFF bipolar cells)². Interestingly, unlike mammalian rods, newt rods are not affected by L-AP4. Similar feedback was observed in carp retina where L-AP4's effect was evaluated by quantifying the frequencies and amplitudes of mEPSPs recorded from a **cone** horizontal cell³. L-AP4 reduces mEPSP frequency, indicating a presynaptic effect and a reduced Ca^{2+} influx in the cones. In this case, the reduced Ca^{2+} current does not result from direct modulation of the Ca^{2+} conductance, but from modulation of the K^+ conductance. Group III receptors contribute to autofeedback also in salamander, however group II receptors play the major role^{4,5}.

The best candidate for the group III receptor in photoreceptor terminals is mGluR8. Its mRNA has been shown in photoreceptors⁶ and the protein has been localized to rat rod and cone terminals at their presynaptic membrane^{1,7,8}. Although a newer study in mouse did not find mGluR8, nor did it find mGluR4, or mGluR7 in the outer plexiform layer⁹, it is possible that the antibodies used by Quraishi et al are not as sensitive as those used by Koulen et al.

A major problem with studying lower vertebrates is that the appropriate reagents (such as antibodies, agonists, or antagonists) needed to study their receptors have not been developed, so experimenters must assume that the receptors behave similarly to their homologous mammalian counterparts. Although specific examples of different pharmacological profiles have been illustrated², such assumptions are often justified because recordings from retinal cells in lower vertebrates are relatively easy and reliable, allowing more thorough experimental protocols.

1.2 Postsynaptic mGluRs in horizontal cells

Immunocytochemical experiments show that rat horizontal cells express mGluR8⁸, but there is no corroborating physiological evidence. Interestingly, electron microscopy shows that only one of the two horizontal cell processes in a triad stains for mGluR8. This suggests that the cone's input at the two horizontal cell processes (each originating from a different horizontal cell) may be modulated differently. The contribution of mGluRs to the signal processing in horizontal cells has been elaborately studied in lower vertebrates, particularly in catfish. Consistent with the findings in rat, in catfish, an agonist for mGluR group III, L-AP4, but not for groups I and II, reduces the current of an inwardly rectifying K⁺ channel (IRK⁺)¹⁰. This response is dependent on phosphorylation by a cGMP-dependent kinase. In other studies IRK⁺ current is shown to contribute to the dark resting potential in horizontal cells, and modulation of this current by glutamate is shown to speed up the hyperpolarizing light response¹¹. In mudpuppy, L-AP4 affects a horizontal cell's responses to spot versus annulus stimulation differently. This differential result has been interpreted to indicate an increase of the apparent electrical coupling between horizontal cells¹². Since this coupling may be a consequence of gap junction phosphorylation by mGluR-activated kinase, the group III mGluRs (possibly mGluR8) located on horizontal cells may serve to shape the light response of these cells.

Another study on catfish horizontal cells found yet another effect. In this study, agonists for mGluR groups I and III increase the sustained calcium current¹³. An attempt to identify the receptors gave positive staining for mGluR1 α , mGluR5, and mGluR6¹⁴. In chick horizontal cells, positive immunostaining is seen for mGluR1 and mGluR5¹⁵. However, since tissue lacking these receptors cannot be tested, these immunostainings may be nonspecific.

1.3 Postsynaptic mGluRs in bipolar cells

1.3.1 ON bipolar cells—ON bipolar cells comprise nearly three-quarters of all bipolar cells, and they hyperpolarize to glutamate released continuously during darkness and depolarize to light onset. Experiments with the glutamate analog L-AP4 have demonstrated the existence of a novel type of glutamate receptor in these cells¹⁶. This receptor was shown to couple to a G protein cascade¹⁷. Within a few years the receptor was cloned and termed mGluR6, a member of the group III metabotropic glutamate receptor family¹⁸. Evidence that supports the key role of mGluR6 in the light signaling of ON bipolar cells includes its localization, the effect of its deletion in a mouse model, and the effect of spontaneous mutations in mice and humans.

1.3.1a. mGluR6 is restricted to the dendritic tips of ON bipolar cells and is required for their light response: Immunohistochemistry and electron microscopic studies localize mGluR6 specifically to the dendritic tips of all types of ON bipolar cells¹⁹⁻²¹. A study conducted in primate retina showed that, within the dendritic tips of ON cone bipolar cells, mGluR6 clusters about 400 nm away from the release site and not directly under it, placing these receptors at a distance similar to that of OFF cone bipolar dendritic receptors²⁰. This restricted expression of mGluR6 is developmentally regulated as these receptors are expressed throughout the bipolar soma early in retinal development, gradually increase in the outer plexiform layer (OPL) around the time of eye opening (day 14), and are finally confined to the OPL by day 28¹⁹.

The first direct evidence for the critical role of mGluR6 in ON bipolar cells came from a study showing that mice lacking mGluR6 lack the ERG b-wave, the wave that indicates ON bipolar cell activity²². Consistent with this data, spontaneous mutations in the mouse *Grm6* gene (*nob3* and *nob4*) lead to negative ERG waveforms and visual behavior

abnormalities²³. Similarly, in human, certain mutations in the GRM6 gene lead to the autosomal recessive complete form of congenital stationary night-blindness (arCSNB)^{24,25}.

1.3.1b. The mGluR6 cascade and its dual role in the ON bipolar cell pathway: A key feature of the photoreceptor to ON bipolar synapse is the reversal of the signal sign from light-induced hyperpolarization in photoreceptors to depolarization in ON bipolar cells. Crucial to this 'sign inverting' synapse is mGluR6 and its cascade (Fig. 3). mGluR6 couples strongly to $G\alpha_o$, the G protein that is critical for the ON bipolar cascade²⁶⁻²⁸. Activation of mGluR6 by glutamate produces activated $G\alpha_o$, particularly $G\alpha_{o1}$ ²⁹, and this in turn leads to closure of TRPM1, a non-selective cation channel that is presumably constitutively active^{30,31}. $G\alpha_{o2}$, another splice variant of $G\alpha_o$, improves the sensitivity of the response³². Several other proteins that appear to play a modulatory or scaffolding role in this cascade include: G β 5, RGS11, RGS7, R9AP, Ret-PCP2, nyctalopin, and possibly Ret-RGS1 (Fig 3)³³⁻³⁵.

Besides playing a direct role in initiating the signaling cascade by binding to glutamate, mGluR6 also has an important function in the post-synaptic localization of several cascade proteins. mGluR6 interacts with the heterotrimeric complex of RGS11/G β 5/R9AP and is required for the proteolytic stability of this complex³⁴. mGluR6 also interacts with a scaffolding protein, nyctalopin, that interacts with TRPM1³⁶. Thus, it is conceivable that this mGluR6/ nyctalopin/ TRPM1 ternary complex leads to the compartmentalization of cascade elements to the dendritic tips of the ON bipolar cells, enabling the fast kinetics of signal transmission. The exact mechanism and role of synaptic activity in this process awaits further research.

1.3.1c. Targeting of mGluR6 to the ON bipolar dendritic tips: Given that mGluR6 is positioned ideally to sense glutamate released by photoreceptors and since it is responsible for the correct targeting of certain signaling complexes, it is important to understand the mechanism that targets mGluR6 itself. Localization and surface expression of many neurotransmitter receptors are governed by the receptor's interaction with proteins that have pdz domains. These proteins help concentrate the transduction cascade components into tight complexes that enable a fast response. They may also play an important role in receptor cycling and thereby may regulate synaptic strength and neuronal function. Two pdz domain-containing proteins, GRIP and syntenin, have been shown to interact with mGluR6 in *in vitro* assays³⁷. However, whether or not they coexist with mGluR6 in ON bipolar cells and play a role in the ON pathway remains to be seen. In contrast, other pdz domain-containing proteins, such as Homer1 and ProSap family members, have been seen at the ON bipolar dendritic tips, but no interactions between these proteins and mGluR6 are known³⁸.

The specific targeting of mGluR6 alters after photoreceptor degeneration or when mutations silence the signaling from photoreceptors to bipolar cells. For example, during the early stages of photoreceptor degeneration in rats with retinal dystrophy, mGluR6 expression is diffuse and is no longer restricted to the dendritic tips¹⁹. Similarly, mutations in bassoon, a presynaptic protein present on synaptic ribbons, or in presynaptic calcium channels (Cacna1f) result in reduced immunostaining for several postsynaptic proteins including mGluR6. In contrast, mutations of the ON bipolar cascade elements (TRPM1, RGS7, RGS11, nyctalopin, or $G\alpha_o$) do not significantly affect the localization of mGluR6^{27,31,34,35,39}. Taken together, these studies suggest that the specific postsynaptic distribution of mGluR6 in the dendritic tips requires a signal from intact photoreceptors and that disruption of the photoreceptor to bipolar synaptic complex hinders the normal localization of mGluR6.

1.3.1d. mGluR6 deletion or mutation leads to molecular and functional defects in the ON bipolar cell:

Under light microscopy, the general structure of the retina and the ON bipolar cells in mGluR6 knockout mice appear normal^{22,40}. However, ultrastructural analysis of nob4, a mouse with a mutation in mGluR6, shows a reduced number of invaginating rod bipolar dendrites³⁴. Moreover, expression of the cascade elements that normally localize at the dendritic tips is affected. For example, in nob4 mouse, Gβ5, RGS7, RGS11, R9AP, and TRPM1 are either down-regulated or redistributed^{34,41}. In mGluR6-null mice, although this ultrastructural analysis has not been done, several proteins, including Gβ5, RGS11, and TRPM1, fail to concentrate in the dendritic tips of the ON bipolar cell (unpublished data). Further abnormalities are found in the dendrites of the rod and ON cone bipolar cells as they contain ectopic synaptic ribbons. Moreover, the dendrites of ON cone bipolar cell express mGluR7, a receptor that is typically restricted to the axon terminals⁴². At the functional level, mice lacking mGluR6 lack the ERG b-wave, and have impaired pupillary responses, optokinetic nystagmus, and behavioral responsiveness to light⁴³.

1.3.1e. Genomic organization and splice variants: The human mGluR6 gene consists of 16,742 bp that encompass 10 exons separated by 9 introns, and it encodes for an 877 amino acid protein⁴⁴. A 9.5 kb upstream genomic region comprises the regulatory elements important for cell-specific and developmentally-regulated expression of the protein⁴⁵. Rodent and human retina also express alternatively spliced variants of mGluR6 that code for truncated isoforms. The alternate splice variant in mouse and rat (mGluR6b), and the two splice variants in human (hmGlu6b, hmGlu6c) are predicted to code for proteins containing 545, 508, 425 and 405 amino acids, respectively⁴⁶. The predicted truncated protein in these species contains the N-terminus extracellular domain of the receptor, and this could be secreted to act as a physiological regulator of glutamate-dependent synaptic signaling. However, its role in the ON bipolar cascade is yet to be elucidated.

1.3.1f. ON bipolar cells also express Group I mGluRs: Although mGluR6 is the dominant glutamate receptor found in bipolar cells, other metabotropic receptors have been seen. Rod bipolar cells in rat and mouse, and cone bipolar cells in chicken express mGluR1a and mGluR5a in their dendrites⁴⁷⁻⁴⁹. ON mixed-input bipolar cell processes in fish also express mGluR1a and there, the receptor appears to serve a modulatory role^{50,51}. However, it is not known if Gα_{o1} mediate the response, or if these receptors couple to Gα_{o2} or Gα_{i2}, G-proteins whose mRNA has been seen in bipolar cells^{28,29}. Furthermore, the downstream effectors or function is still unclear.

1.3.2. OFF bipolar cells—This class of bipolar cell senses glutamate mainly via AMPA/Kainate receptors. Joselevitch et al. (2007) reported that goldfish OFF bipolar dendrites stain for mGluR7, and Koulen et al. (2002) found that about 1 out of 10 rat OFF bipolar dendrites expresses mGluR8a.

2. The second synaptic layer: synapses from bipolar cells to third order cells

The second synaptic layer contains numerous and diverse types of synapses that connect about 10 types of bipolar cells to about 20 types of amacrine and 20 types of ganglion cells. Similar to the photoreceptor, the bipolar axon terminal uses a presynaptic ribbon that coordinates glutamate release onto postsynaptic amacrine and ganglion cells at a structure called a “dyad”. This synaptic layer also contains numerous types of feedback synapses from amacrine cells onto bipolar and ganglion cells. Thus, in this layer, bipolar cell axon terminals are clearly presynaptic, ganglion cell dendrites are postsynaptic, but amacrine cell processes can be presynaptic, postsynaptic, or both in the same compartment (Fig. 1).

2.1 Presynaptic mGluRs in bipolar cells

As with the autofeedback system of the photoreceptor, mGluRs, particularly those from group III, serve as autoreceptors at the synapse from the bipolar axon terminal to the ganglion cells. Support for this comes from work on salamander retina where recordings were taken from OFF ganglion cells to avoid the inevitable effect of modulating the mGluR6 of ON bipolar cells^{4,52}. These studies showed that L-AP4 reduces the frequency of spontaneous mEPSPs and even reduces the response evoked by turning off the light. However, the autofeedback in these bipolar cells is complicated by the precise parameters of the light stimulus. L-AP4 does not affect the response to turning off a dim or a strong light, but only a middle intensity light. Furthermore, L-AP4 may enhance the response to turning off a short light stimulus, but not to turning off a long light stimulus. Though a precise function for glutamate autoreceptors cannot be concluded at this time, the general function is clear. Autoreceptors reduce glutamate release from bipolar cell terminals, perhaps to shape the light response and to protect the retina from toxic glutamate levels.

The receptor at the bipolar cell axon terminal is most likely mGluR7. The mRNA for this receptor was found in rat bipolar cells^{53,54}. Moreover, the protein was localized to the bipolar cell presynaptic membrane, a staining that disappeared in the mGluR7-null retina⁵⁵. Examining mGluR7 localization in bipolar cells led to three interesting observations: (i) mGluR7 localizes to ON and OFF cone bipolar cells, but not to rod bipolar cells; (ii) the receptor localizes to only one side of the dyad, i.e., it opposes only one of the two postsynaptic dendrites; and (iii) when ON cone bipolar cells lose mGluR6, their dendrites gain mGluR7 staining near the abnormally present ectopic ribbons⁴². The localization of mGluR7 to only one side may have far reaching consequences in signal transmission, because the two postsynaptic elements may get input that is only partially rather than completely correlated. Although mGluR7 localization to the bipolar cell axon terminals is quite certain, it does not fit the physiological finding that the presynaptic receptor is more sensitive than mGluR7 is known to be⁴. However, it is possible that receptors in native tissue have different sensitivities than those in cell lines.

2.2 Pre- and postsynaptic mGluRs in amacrine cells

There are over 20 types of amacrine cells in the retina, and they house most of the mGluR types (Table 1 and Fig. 1). This suggests that mGluRs play a diverse role in signal processing, but difficulty in identifying amacrine cell types constrains current knowledge of their function. The action of mGluRs on amacrine cells has mostly been studied in lower vertebrates and chick. A common outcome of stimulating amacrine cells with group I agonists is a slow increase of intracellular calcium, most likely via the PLC-IP3 pathway. Examples to the contrary are given below. The main effect of agonists of the other groups is also a decrease in release, but probably by a different chemical pathway (Fig. 2). A short review of mGluRs in amacrine cells was published recently⁵⁶. Some examples of the physiological effects mediated via mGluRs in amacrine cells are given below.

2.2a. Effect of Group I mGluRs—Goldfish ON bipolar cells have particularly large axon terminal, making them an easy target for studying synaptic properties. These bipolar cells, as all bipolar cells in all species, form reciprocal synapses with GABAergic amacrine cells. Recordings from these cells showed that when they release glutamate over a long time period, the GABAergic feedback that the reciprocal amacrine cell synapse exerts on them is enhanced⁵⁷. This effect of glutamate was attributed to mGluR1, which presumably stimulates PLC, increases intracellular calcium, and hence increases GABA release. This enhanced inhibition may contribute to slow adaptation.

Elevation of calcium was also seen in chick amacrine cells in culture⁵⁸, but the downstream effects are diverse. In some cases, subsequent to release from stores, mGluR5 further elevates calcium by increasing the L-type calcium current⁵⁹. And while mGluR5 tends to elevate calcium, mGluR1 reduces this elevation¹⁵. Pathways further diverge because calcium has multiple effects in cells, so the downstream effects of calcium elevation are different in different cells.

Recordings from isolated calmodulin-positive wide field amacrine cells in white bass showed that glutamate or mGluR1 agonists reduce the response of these cells to applied GABA. This effect is slow, and is likely mediated via calcium as follows: mGluR1 $\rightarrow$ Gq $\rightarrow$ PLC $\rightarrow$ IP₃R $\rightarrow$ Ca²⁺ $\rightarrow$ Ca/calmodulin $\rightarrow$ calcineurin $\rightarrow$ dephosphorylates GABA_A receptor and reduces its sensitivity⁶⁰. Rats display a similar modulation of the GABA_C receptor⁶¹. In contrast, mGluR5 in cultured amacrine cells enhances GABA_A receptor current, probably due to phosphorylation by protein kinase C⁵⁸.

2.2b. Effect of Group II mGluRs—A unique and interesting type of amacrine cell is the cholinergic cell (also called the “starburst”). Starburst amacrine cells are divided into two subtypes, the ON and the OFF. Starburst cells draw much attention in the retina field because they release both GABA and acetylcholine; they respond preferentially to light or dark bars that move centrifugally; and they are an essential component of the circuit that constructs directional selectivity in the directional selective ganglion cells⁶². Relevant to mGluRs, these amacrine cells express mGluR2^{40,63,64}. When a group II mGluR agonist is applied to the retina, the directional selective ganglion cell loses its directional selectivity, and this is probably due to inhibition of GABA release. When a group II antagonist is applied, this cell’s response to its preferred direction is reduced⁶⁵. It appears therefore, that glutamate not only provides the major light signal to the starburst and ganglion cells (via ionotropic receptors), but that it also modulates the starburst output (via mGluR2) to further increase the ganglion cell’s response to the preferred direction.

2.2c. Effect of Group III mGluRs—Examples of Group III mGluRs in amacrine cells are few. In one example, recordings from OFF and ON-OFF ganglion cells show that applying mGluR8-specific agonist, DCPG, greatly reduces their OFF response, but only slightly affects the ON response of ON or ON-OFF ganglion cells⁹. This effect appears presynaptic to ganglion cells, i.e., it occurs either on bipolar cell terminals or on amacrine cells. However, because mGluR8 is probably not localized to ON bipolar cells, DCPG most likely affects the ganglion cells through the amacrine cells. This agrees with the observation in other systems, including chick cultured amacrine cells, that application of L-AP4 suppresses the calcium current and consequently transmitter release⁶⁶.

2.3 Postsynaptic mGluRs in ganglion cells

Ganglion cells collectively (i.e., different types and different species) express most of the mGluR proteins (see Table 1). Experiments designed to investigate the mGluRs in these cells without interference from the network generally use acutely cultured or isolated ganglion cells. In *Xenopus*, **group I** mGluRs reduce voltage-activated calcium current, in particular the L-type⁶⁷. In mouse, however, this group (and possibly also group III) may enhance or reduce N channel current⁶⁸. In rat, mGluR1 decreases K⁺ current; this in turn increases the input resistance of the cells, depolarizes them and increases their spontaneous activity⁶⁹. Calcium is also elevated, but since TTX blocks this elevation, this elevation must be due to the depolarization.

Group II (specifically mGluR2) generally acts to increase low voltage-activated calcium current salamander,⁷⁰ rat,⁷¹ but it may reduce N-type calcium current⁷². In salamander,

Group III reduces L-type calcium current, an effect that interestingly appears to be mediated by the IP₃ receptor⁷⁰.

3. mGluRs and retinal disorders

Certain mutations in the GRM6 gene (encoding mGluR6 protein) lead to autosomal recessive congenital stationary night blindness (arCSNB) that is characterized by complete loss of night vision²⁵. This defect is consistent with the absence of the ERG b-wave under dark adapted conditions in mice lacking mGluR6. In families with arCSNB, a total of 10 disease-causing sequence variations have been observed in the GRM6 gene. These include mis-sense, frame shift and non-sense mutations⁷³. The mis-sense mutations (P46L, G58R, G150S, I405T, C522Y, and E781K) in the GRM6 gene have been analyzed in HEK cells⁷³ or in neuronal cultures⁷⁴, and most of them seem to affect the trafficking of the mGluR6 receptor to the plasma membrane. One mutation in rat mGluR6 (E775K, corresponding to E781 in human) produces an inability of mGluR6 to couple to G α_o , but it does not alter its cell surface expression or its affinity for glutamate.

GRM8, the gene for the mGluR8 protein, is considered to be a strong positional candidate for retinitis pigmentosa 10 (RP10). This is because the GRM8 gene is an exceptionally large (~1Mb) gene that maps to the same region as the mutation that causes RP10, and because it is expressed in retinal photoreceptors⁷⁵ (see Table 1).

A few studies have suggested that mGluRs play some trophic or neuro-protective role; however, evidence for this role is rather indirect. First, in juvenile rats, subcutaneous administration of DL 2-amino-3-phosphono-propionic acid (AP3), a mGluR antagonist, has retinotoxic effects⁷⁶. The authors suggest that glutamate triggers a number of second messenger cascades that may be vital for growth, development and survival of the neurons, and these are inhibited by DL-AP3. However, this study does not conclusively demonstrate the mechanistic basis of the effect, i.e., whether the effect is mediated via a generalized expression of DL-AP3 sensitive receptors throughout the retina or via their expression in cells that play a supportive role for developing retina. Second, in rat retina, activation of mGluRs reduces the toxic effect of N-methyl-D-aspartate (NMDA)⁷⁷. Third, neuronal injuries lead to adaptive changes in mGluR expression that resemble the pattern in development. Developing ganglion cells express transcripts for all types of mGluRs, and several of these downregulate in adulthood. Upon neuronal injury or ganglion cell axotomy, this developmental downregulation appears to reverse. For example, mGluR6 mRNA, which is not present in adult RGCs, reappears after axotomy; similarly, mGluR1 and -7 are more abundant in axotomized retina than in control⁷⁸.

4. Concluding remarks

While the function of mGluR6 is known, largely due to its crucial and direct role in the ON bipolar cell cascade, the precise role of other mGluRs is only beginning to be elucidated. This understanding will greatly benefit from newly developed specific ligands and novel approaches to study receptor biology. The large diversity of mGluRs in retina, with almost all cell types expressing these receptors at the pre- and/ or post-synaptic sites, suggests multitude of functions in governing responses to glutamate, ranging from feedforward signaling to modulatory or fine-tuning responses. These multitude of functions most likely also occur in development and in diseased tissues. Consequently, better understanding of the mGluR pathways and their role in development and disease in retina is expected to help design therapeutic strategies for retinal diseases in future.

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Fig. 1. Localization of mGluRs in the retinal circuits of the rodent retina

All but mGluR3 proteins are expressed in this retina. A single cell type often expresses more than one mGluR. However, localization of mGluRs has often been assigned to a class rather than a single type of cell, so many of the seemingly co-expressed receptors may segregate to cell types. The rod bipolar cell (RB) likely represents a single cell type; the OFF cone bipolar cell (Off BP) and the ON cone bipolar cell (On BP) each contains 4-5 types. The cholinergic amacrine cells (Chat) have two subtypes (ON and OFF). The prototypic amacrine cell (AC) and the ganglion cell (GC) in this scheme represent about 20 types of ON and OFF for each cell class. Lightly shaded cells are ON cells (depolarize to light) while darkly shaded cells are OFF (hyperpolarize to light). AC and GC are shaded in a medium tone to indicate that they represent both ON and OFF types. Photoreceptors (Rod and cone) and horizontal cells (HC) are colored yellow because, although they hyperpolarize to light, they are neither ON nor OFF. Colored areas at the various terminals indicate localization of mGluRs and the color key shows which particular type of mGluR. Arrows designate general information transfer via feedforward and feedback synapses.

Fig. 2. Similarity and diversity of specific mGluR pathways in the retina

All mGluRs modulate either neurotransmitter release or channel current. When the receptor is located presynaptically, it acts as an autoreceptor that decreases glutamate release. When it is located postsynaptically, it acts to either increase or decrease neurotransmitter release, depending on the type and/or state of the cell. Correspondingly, modulation of the channel current can go either way, typically via phosphorylation or dephosphorylation. *¹: this effect was attributed to group I and not necessarily to mGluR5. *²: modulation of the L-type Ca²⁺ current was specifically shown as a shift of its activation curve. PLC, phospholipase C; IP₃R, inositol 1,4,5-tri-phosphate receptor; PKC, protein kinase C; GP, an unidentified G-protein; PKG, cGMP-dependent protein kinase. Arrows indicate either a direct or indirect effect.

Fig. 3. Protein complex and mGluR6 cascade in ON bipolar cells

Glutamate released by photoreceptors binds to mGluR6 receptors on the dendritic tips of ON bipolar cells and activates the G protein G_o. The α subunit of this G protein is activated by binding to GTP and dissociates from the βγ partners. Either α or βγ closes the TRPM1 channel and hyperpolarizes the cell. Several molecules modulate this cascade. These include Ret-PCP2 which may act as a GEFs (guanine nucleotide exchange factor) or GDI (guanine nucleotide dissociation inhibitor); RGS (regulator of G-protein signaling) including RGS7, RGS11, and possibly Ret-RGS; Gβ5, an atypical G protein β subunit; and R9AP, a transmembrane protein associated with anchoring. mGluR6 associates with the RGS11/Gβ5/R9AP heterotrimer and helps stabilize the complex at the dendritic tips. mGluR6 also makes a complex with a scaffolding protein, nyctalopin, which interacts with TRPM1 and thus may help to bring about a fast response. These protein complexes (represented in the oval) are localized to the dendritic tips.

Table 1

Expression and localization of mGluRs in retinas of different species

References for the expression and localization of mGluRs in the cell types of the retina.

Receptor	Molecular species	Photoreceptors	Horizontal cells	Bipolar cells	Amacrine cells	Ganglion cells
mGluR1	mRNA				H95 (rat)	H95; T00 (rat)
	Protein	CP99 ^{*1} ; KG01; SG06 ^{*2} (cat and chicken)	SG06; J07 (chicken and goldfish)	K97 ^{*3} ; SG06 ^{*4} ; J07 (rat, chicken and goldfish)	K97; CP99 ^{*5} ; KG01; SG06 (rat, cat and chicken)	KG01; CP99 (chicken and cat)
mGluR2	mRNA				O93; H95 (rat)	O93; H95 ^{*5} ; T00 (rat)
	Protein		CP99 ^{*6} ; J07 ^{*6} (cat, goldfish)		K96 ^{*7} ; CP99 ^{*8} (rat and cat)	
mGluR3	mRNA			HC 07 (primate)		T00 (rat)
	Protein					
mGluR4	mRNA				H95 (rat)	A94; H95; T00 (rat)
	Protein				K96; Q07 ^{*13} (rat and mouse)	K96 (rat)
mGluR5	mRNA		H95 (rat)	H95 (rat)		T00 (rat)
	Protein	KG01; SG06 ^{*2} (chicken)	KG01; SG06 (chicken)	K97 ^{*3} ; SG06 ^{*4} (rat and chicken)	K97; KG01; SG06 (rat and chicken)	KG01 (chicken)
mGluR6	mRNA			N93; A94; H95 (rat)		T00 ^{*9} (rat)
	Protein			N94; VM97; V00 (rat and monkey); ^{*10}		
mGluR7	mRNA			A94 (rat); H95	A94; H95 (rat)	A94; H95 ^{*5} ; T00 (rat)
	Protein		J07 (goldfish)	B96 ^{*11} ; J07 ^{*5} (rat and goldfish)	B96; Q07 ^{*13} (rat and mouse)	
mGluR8	mRNA	D95 (mouse)		D95 (mouse)	D95 (mouse)	D95; T00 (mouse and rat)
	Protein	K99; KB02 (mouse and rat)	KB02 (mouse and rat)		K99; KB02; Q07 ^{*12} ; I3 (rat and mouse)	K99; KB02 (rat and mouse)

Species tested is in parenthesis.

The starred numbers refer to the information listed below.

^{*1} - receptor was found only in rods;

^{*2} - receptor was found in cones but not rods;

^{*3} - receptor was found in rod bipolar cells and rarely in cone bipolar cells;

- ^{#4} – receptor was found postsynaptic to cones but not rods.
- ^{#5} – receptor was found only in a few cells of this class, postsynaptic to cone bipolar cells;
- ^{#6} – antibody recognized both mGluR2 and 3;
- ^{#7} – receptor was found specifically in cholinergic amacrine cells;
- ^{#8} – receptor was found postsynaptic to cone bipolar cells and in A17 cells postsynaptic to rod bipolar cells;
- ^{#9} – mRNA was found only during development and after axotomy;
- ^{#10} – numerous articles reported expression of this receptor in ON bipolar cells; only the originals are cited;
- ^{#11} – receptor was found in cone bipolar, but not rod bipolar cells;
- ^{#12} – receptor was found in several types of amacrine cells, including the OFF but not the ON Chat.
- ^{#13} localization of mGluR4, 7, 8 were punctate in IPL; puncta of different receptors did not colocalize, but no discrimination between amacrine or ganglion cells was made.

Abbreviations:

A94 53; B96 55; CP99 64; D95 6; HC07 79; H95 54; J07 51; K97 47; K96 63; K99 7; KB02 8; KG01 15; N93 18; N94 19; O93 80; Q07 9; SG06 48; T00 78; VM97 21; V00 20.